

FIG. 1

pBMesterase1

pBluescript II KS+

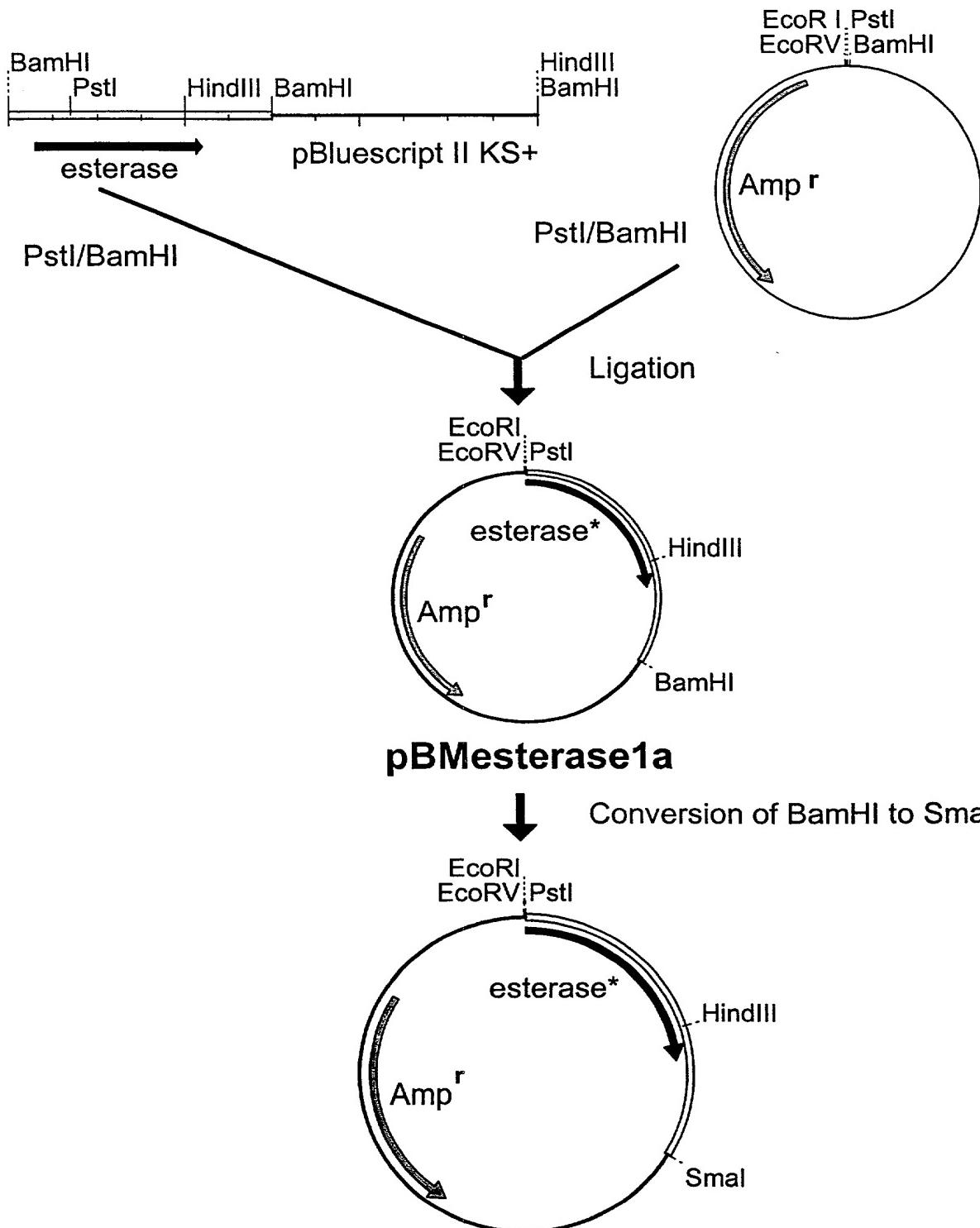
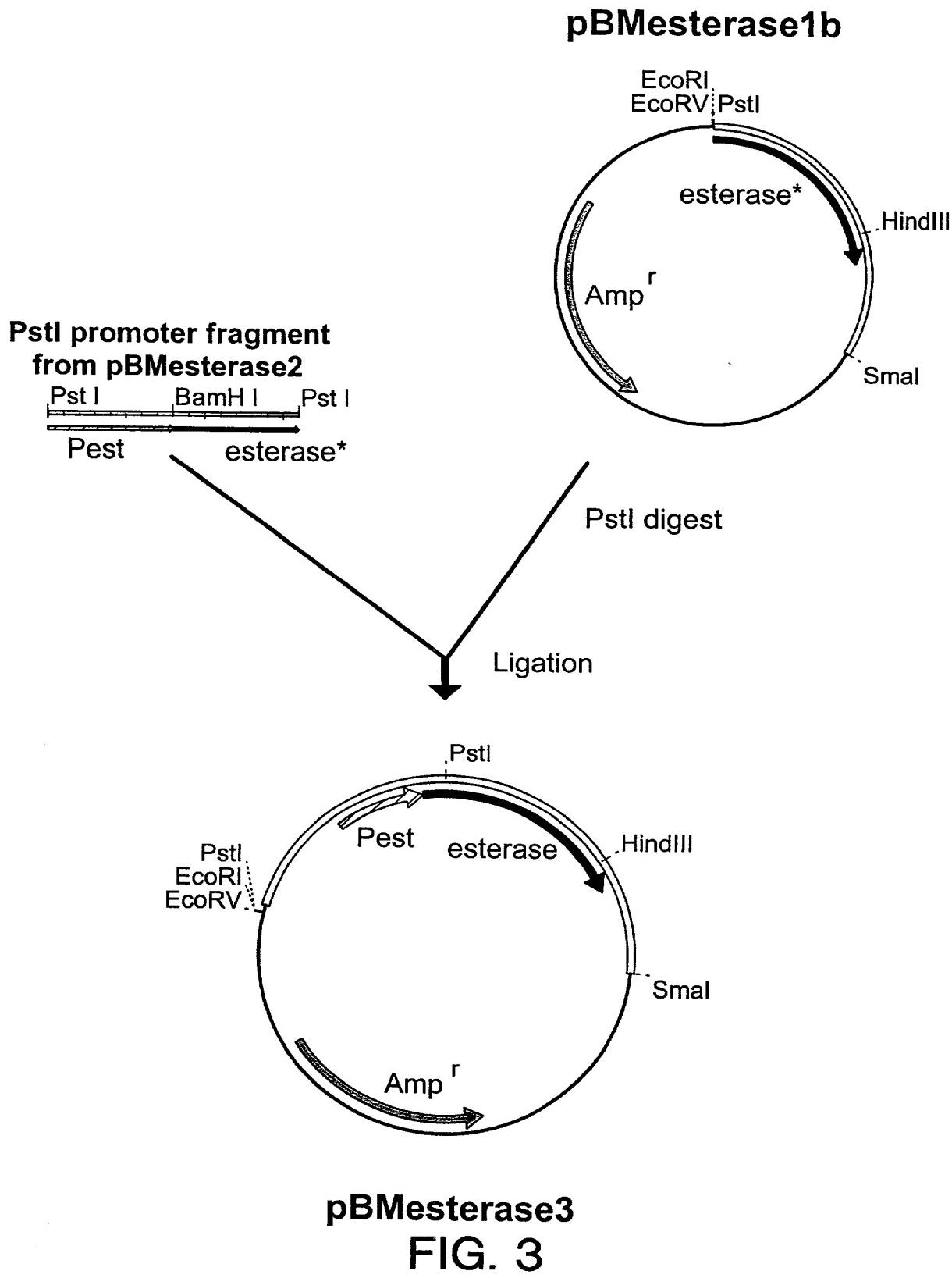
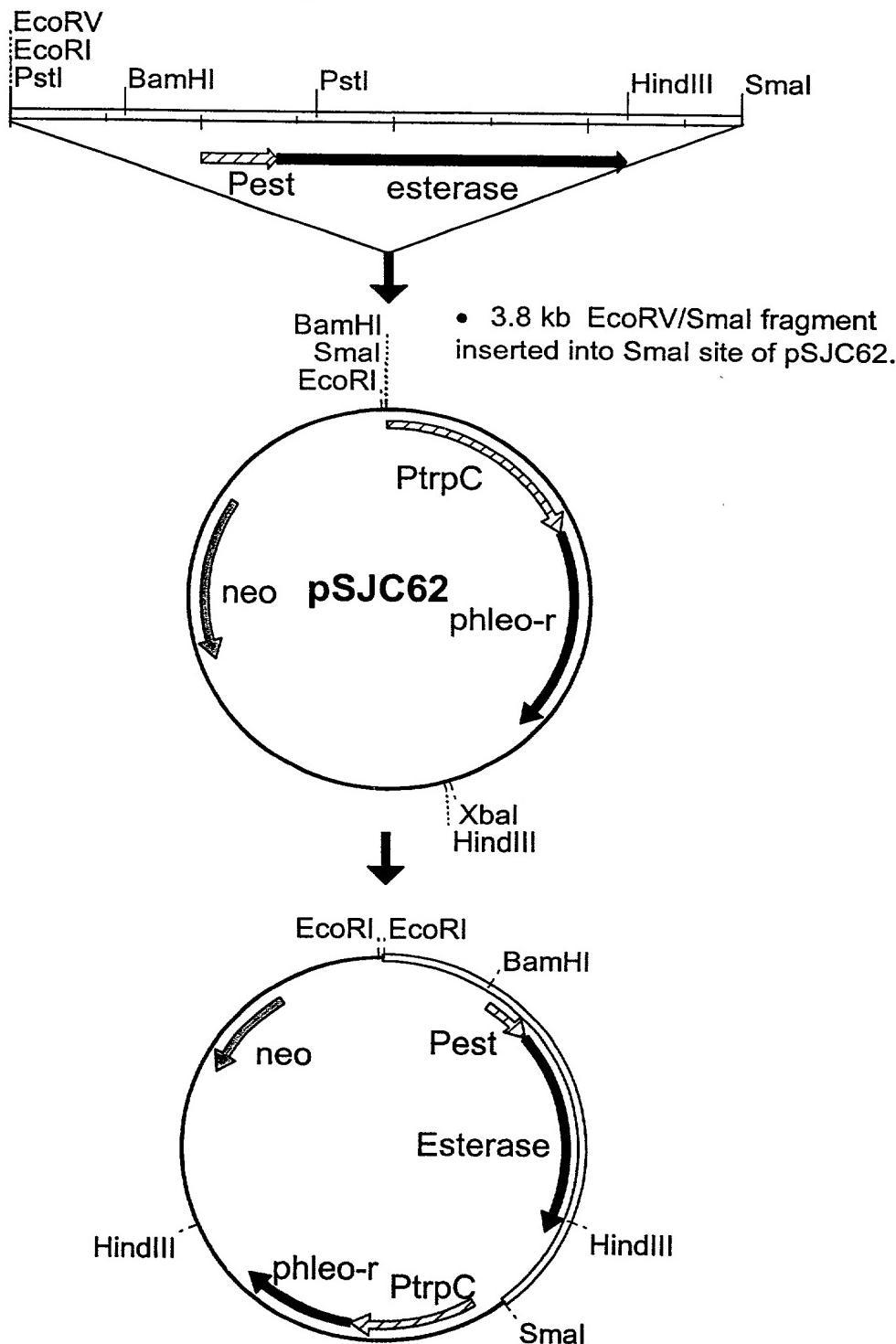


FIG. 2



**Smal/ EcoRV esterase fragment
from pBmesterase3**



pSJC62.3

FIG. 4

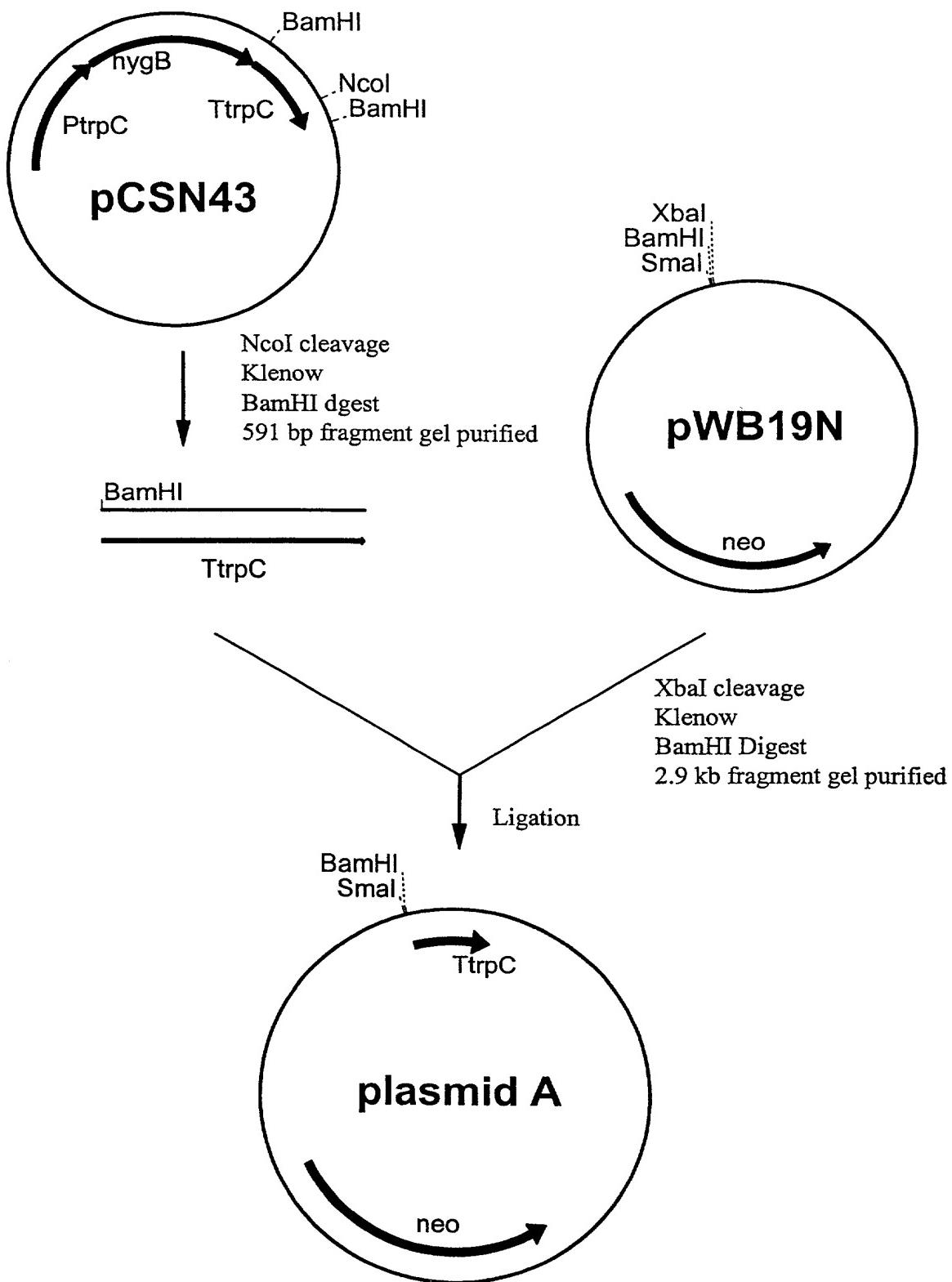


FIG. 5

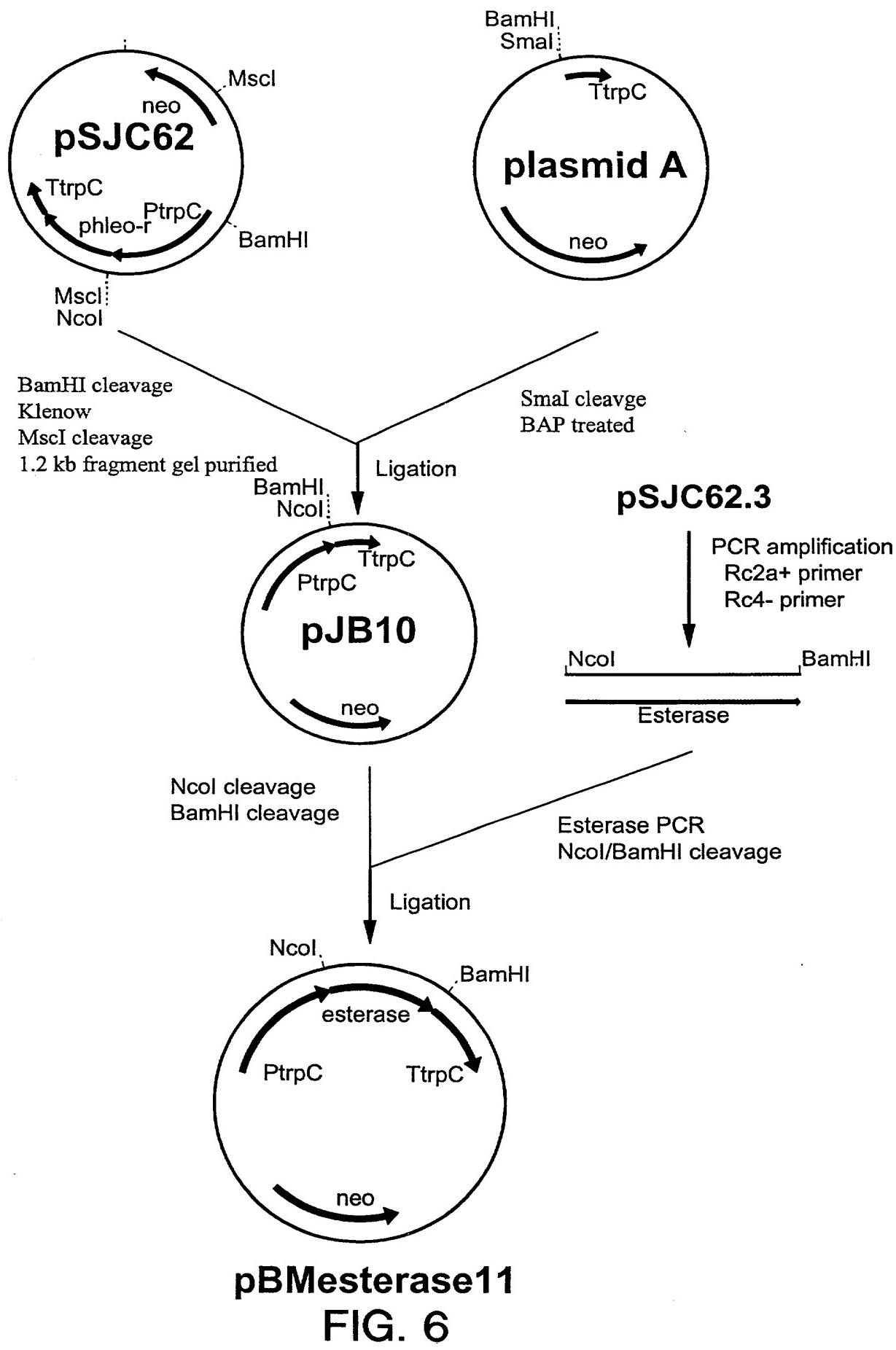


FIG. 6

N-TERMINAL AMINO ACID SEQUENCE

AMINO ACID SEQ.	T N P N E P
REV. TRANSLATION	ACX AAPy CCX AAPy GAPu CC
INVERSE	GGPy TCPu TTX GGPu TTX GT
PROBE	GGPy TCPu TTG GGPu TTX GT
1	
2	A
3	T
4	C

Four 17-mer oligonucleotide probes each with a 32-fold degeneracy were synthesized from the N-terminal amino acid sequence and used to probe a Southern blot of *R.toruloides* DNA.

FIG. 7

RHODOSPORIDIUM ESTERASE cDNA

ATGCTCCTAACCTCTTCACCCCTGCCTCCCTCGCTGCACGCTCCAGCTCGCCTTGCC M L L N L F T L A S L A A T L Q L A F A	70
TCTCCGACCTCCCTCGCCGCCACGAACCCAAACGAGCCCCCTCCCGTGTGACCTC S P T S L V R R T N P N E P P P V V D L	130
GGCTACGCCGCTACCAAGGCTACTTGAACGAGACCGCCGGACTCTACTGGTGGCGCGGA G Y A R Y Q G Y L N E T A G L Y W W R G	190
ATCCGCTACGCCCTGGCTCAGCGCTTCCAGGCTCCTCAGACGCCCGCACGCACAAGGCC I R Y A S A Q R F Q A P Q T P A T H K A	250
GTCCGCAACCGCACTGAGTATGGACCGATCTGTTGGCCGGCTAGCGAGGGAACCAACACG V R N A T E Y G P I C W P A S E G T N T	310
ACCAAGGGCTTGCCGCCCTAGCAACAGCTCGAGCAGCGCCGCAGAACACAGCGTCG T K G L P P S N S S S S A P Q K Q A S	370
GAGGATTGCCCTTCTCAATGTCGTTGCCCGCCGGCTCGTGGAGGGGACAATCTT E D C L F L N V V A P A G S C E G D N L	430
CCCGCTCGTCTACATTACGGAGGTGGCTACGCCCTCGGCCGATGCGAGCACCGGCAGC P V L V Y I H G G G Y A F G D A S T G S	490
GACTTTGCCGCCTCACCAAGCACACGGAACCAAGATGGTCGTTGTAATCTCCAGTAC D F A A F T K H T G T K M V V V N L Q Y	550
CGTCTCGGCAGCTTGGTTCTCGCTGCCAAGCCATGAAGGACTACGGTGTAAACGAAC R L G S F G F L A G Q A M K D Y G V T N	610
GCCGGCTGCTTGACCAGCAATTGCCCTCAATGGGTTCAACAGCACGTCTCGAAGTTC A G L L D Q Q F A L Q W V Q Q H V S K F	670
GGCGGCAACCCGATCACGTTACGATTGGGGCGAGTCTGCAGGCGCAGGGTCCGTTATG G G N P D H V T I W G E S A G A G S V M	730
AACCAGATCATTGCGAACGGCGAACACCGTCAAGGCTCTGGTCTCAAGAACGCCCTC N Q I I A N G G N T V K A L G L K K P L	790
TTCCACGCTGCCATGGCTCCCTCCGTCTCCTCCCTACCAAGCCAAGTACAACCTCCCC F H A A I G S S V F L P Y Q A K Y N S P	850
TTCGCCGAGCTGCTACTCCAACTCGTCTGGCGACAAACTGCACCAAAGCCGCCCTCG F A E L L Y S Q L V S A T N C T K A A S	910
TCCTTCGCTTGCCCTGAAGCTGTCGACGCTGCCGCTCGCTGCCGGCGTGAAGAAC S F A C L E A V D A A A L A A A G V K N	970
TCGGCGGGCGTTCCCGTTGGGTTTGGTCGTATGTCCC GGTCGACGGGACCTTCTTG S A A F P F G F W S Y V P V V D G T F L	1030

FIG. 8A

ACTGAGCGCGCGTCGCTCCTCTGCCAAGGGCAAGAAGAACCTCAATGGCAACCTCTTC 1090
 T E R A S L L L A K G K K N L N G N L F

 ACCGGGATCAACAACCTCGACGAAGGATTCAATTCACTGACGCCACTATTAGAACGAC 1150
 T G I N N N L D E G F I F T D A T I Q N D

 ACGATCAGCGACCAGTCGCAGCGCGTCTCCAGTTGACCGCCCTCCTGCCGGCTCTTC 1210
 T I S D Q S Q R V S Q F D R L L A G L F'

 CCCTACATCACCTCGGAGGAGCGCCAGGCCGTGCGAAGCAGTACCCGATCTCCGACGCG 1270
 P Y I T S E E R Q A V A K Q Y P I S D A

 CCGTCAAAGGGCAACACCTCTCGCATCTCGGCCGTATCGCGGACTCGACCTCGTC 1330
 P S K G N T F S R I S A V I A D S T F V

 TGCCCACCTACTGGACCGCCGAGGCCTCGCTCGCCGCCCACAAGGGCCTTCGAC 1390
 C P T Y W T A E A F G S S A H K G L F D

 TACGCGCCGGCTCACCA CGCGACCGACAAC TCGTACTACATCGGCTCCATCTGGAACGGC 1450
 Y A P A H H A T D N S Y Y I G S I W N G

 AAGAAGTCGGTCTCGTCCAGTCCTCGACGGCGCGCTCGGCGGCTTCATCGAGACG 1510
 K K S V S S V Q S F D G A L G G F I E T

 TTCAACCGAACAAACACGCTGCCAACAGACCATCAACCCCTACTGGCCGACGGTTCGAC 1570
 F N P N N N A A N K T I N P Y W P T F D

 TCGGGCAAGCAGCTCCCTTCAACACGACGAGGGACACCCCTCTCTCCCGCCGACCCG 1630
 S G K Q L L F N T T T R D T L S P A D P

 CGCATTGAGACTTCAAGCTTGACCGACTTGGCACGAGCCAGAACGACCAAGTGCAC 1690
 R I V E T S S L T D F G T S Q K T K C D

 TTCTGGCGTGGTCAATCTCGGTGAACGCGGGTCTC 1726
 F W R G S I S V N A G L

FIG. 8B

GGATCCACCCGAACTCTGTCCCCTTCTGGCTTCCTGCTGTCGCCCATCGCCT 60
 | -- Translation Start -->
 TTCCCGACTGCCGCCATGCTCCTAACCTCTCACCCCTCGCCTCCCTCGCTGCGACGCT 120
 M L L N L F T L A S L A A T L
 | - Mature peptide ->
 CCAGCTCGCCTTGCCCTCGACCTCCCTCGTCCGCCACGAACCCAAACGAGCCCCC 180
 Q L A F A S P T S L V R R T N P N E P P
 TCCCCTCGTCGACCTCGGCTACGCCCGCTACCAAGGCTACTTGAAACGAGACC CGCCGGACT 240
 P V V D L G Y A R Y Q G Y L N E T A G L
 CTACTGGTGGCGCGGAATCCGCTACGCCCTCGGCTCAGCGCTTCCAGGCTCCAGACGCC 300
 Y W W R G I R Y A S A Q R F Q A P Q T P
 CGCGACGCACAAGGCCGTCCGCAACGCGACTGAGTATGGACCGATCTGTTGGCCGGCTAG 360
 A T H K A V R N A T E Y G P I C W P A S
 CGAGGGAACCAAACACGACCAAGGGCTTGCCGCCCTAGCAACAGCTCGAGCAGCGCGCC 420
 E G T N T T K G L P P P S N S S S S A P
 GCAGAAACAGCGTCGGAGGATTGCCCTTCCTCAATGTCGTTGCCCGCCGGCTCGTG 480
 Q K Q A S E D C L F L N V V A P A G S C
 CGAGGGCGACAATCTCCCGTCCCTCGTCTACATTACGGAGGTGGCTACGCCCTCGGCGA 540
 E G D N L P V L V Y I H G G G Y A F G D
 TGCGAGCACCGGCAGCGACTTGGCCCTTCACCAAGCACACGGGAACCAAGATGGTCGT 600
 A S T G S D F A A F T K H T G T K M V V
 TGTAAATCTCCAGTACCGTCTCGGCAGCTTGGCTTCCTCGCTGGCCAAGCCATGAAGGA 660
 V N L Q Y R L G S F G F L A G Q A M K D
 [---- Intron #1 -----]
 CTACGGTGTAAAGAACGCCGGCTTGCTTGACCGAGTTCCCGCATGATAACCCGCC 720
 Y G V T N A G L L D Q
 -----]
 ACCTTTGACTCATGCTGACGCCCTCCCGCTCGCAGCAATTGCCCTCAATGGTTCA 780
 Q F A L Q W V Q
 ACAGCACGTCTCGAAGTTGGCGGCAACCCGATCACGTTACGATTGGGGGAGTCTGC 840
 Q H V S K F G G N P D H V T I W G E S A
 [---- Intron #2 -----]
 AGGCGCAGGGTCCGTTATGAACCAGATCATTGCGAACGTGAGCCACCCGAACCGATCTCC 900
 G A G S V M N Q I I A N
 -----]
 AGCCGACTTTCCCCCCCCCCCCCGCTGACCTCCCTCGTCTTGCAAGGGCGGCAACA 960
 G G N T
 CCGTCAAGGCTCGGTCTCAAGAAGCCCTTCCACGCTGCCATGGCTCCCGTCT 1020
 V K A L G L K K P L F H A A I G S S V F
 TCCTCCCTACCAAGCCAAGTACAACCTCCCTCGCCGAGCTGCTCTACTCCAACTCG 1080
 L P Y Q A K Y N S P F A E L L Y S Q L V

FIG. 9A

CTGGCGGCTCGCTGCGGGCGGTGAAGAACTCGGCGGCGTCCCGTTGGTTGGT
 A A L A A A G V K N S A A F P F G F W S 1200
 CGTATGTCCCCGGTCGACGGGACCTTCTTGACTGAGCGCGCGTCCCTCTCGCCA
 Y V P V V D G T F L T E R A S L L L A K 1260
 [---- Intron #3 ----]
 AGGGCAAGAAGAACCTCAATGGCGTGCAGCAGCTTCACCGGGATCAACAACCTCGACGAAGATGAG
 G K K N L N G 1320
 -----] [---
 GACACTGTCGACCGGCTCGCAGAACCTCTTACCGGGATCAACAACCTCGACGAAGATGAG
 N L F T G I N N L D E G 1380
 ---- Intron #4 ----] }
 GTTCCCGTCGACGGCTCTGTTGCCAGCGAGACTGACTTGTCTTTCGCAAGATTACG 1440
 ATTCAATTCACTGACGCCACTATTCAGAACGACACGATCAGCGACCAGTCGCAAGCGCT
 F I F T D A T I Q N D T I S D Q S Q R V 1500
 CTCCCAGTTCGACCGCCTCCCGCCCTCTTCCCTACATCACCTCGGAGGAGCGCCA
 S Q F D R L L A G L F P Y I T S E E R Q 1560
 GGCGTGCAGAACGAGTACCCGATCTCCGACGCCGGCTCAAAGGGCACACCTCTCG
 A V A K Q Y P I S D A P S K G N T F S R 1620
 [---- Intron #5 ----]
 CATCTCGGCCGTCACTCGGGACTCGACCTTCGTGTGCGTCCCGTCTCCGAGT
 I S A V I A D S T F V 1680
 -----]
 ATTCCGCTGACTTCCCGCTTGCCCGAGCTGCCGACCTACTGGACCGCCGAGGCCTCG
 C P T Y W T A E A F G 1740
 GCTCGCCGCCACAAGGGCTCTCGACTACGCCGGCTCACCGCGACCGACAACT
 S S A H K G L F D Y A P A H H A T D N S 1800
 CGTACTACATCGGCTCCATCTGGAACGGCAAGAAGTCGGTCTCGTCCAGTCCTCG
 Y Y I G S I W N G K K S V S S V Q S F D 1860
 ACGGCGCGCTGGCGCTTCATCGAGACGTCACCCGAACAACAACGCTGCCAACAGA
 G A L G G F I E T F N P N N N A A N K T 1920
 CCATCAACCCCTACTGGCCGACGTTGACTCGGGCAAGCAGCTCCCTCTCAACACGACGA
 I N P Y W P T F D S G K Q L L F N T T T 1980
 CGAGGGACACCCCTCTCCCGCCGACCCGCGCATCGTTGAGACTCAAGCTTGACCGACT
 R D T L S P A D P R I V E T S S L T D F 2040
 TTGGCACGCCAGAACCAAGTGCAGCTCTGGCGTGGGTCATCTCGGTGAACGCCGG
 G T S Q K T K C D F W R G S I S V N A G 2100
 GTCTCTAGGCCTTCCGACTTCCTCGTTCTCGTTGTTATTCTTGCAAGTTC
 L * 2160
 CGTTGTATCGGCCATTGTGCGTAGCTCACTCGAGTATAGACGTTGGCAAGTGCAGAAA 2220

FIG. 9B

TRN 2-1738 RHODOSPORIDIUM ESTERASE cDNAs

↑ Translation Start ↑ Mature Peptide
MLLNLFLASLAATLQLAFASPTSLVRRTNPNEPPPVVDLGYARYQGYLNETAGLYWWRG
IRYASAQRFQAPQT PATHKAVRNATEYGPICWPASEGTNTTKGLPPPSNSSSAPQKQAS
EDCLFLNVVAPAGSCEGDNLPVLVYIHGGGYAFGDASTGSDFAAFTKHTGTMVVVNQY
RLGSFGFLAGQAMKDYGVTNAGLLDQQFALQWVQQHVSKFGGNPDHVTIWGESAGAGSVM
NQIIANGGNTVKALGLKKPLFHAAIGSSVFLPYQAKYNSPFAELLYSQLVSATNCTKAAS
SFACLEAVDAAALAAAGVKNSAAPPFGFWSYVPVVDGTFLTERASLLLAKGKKNLNGNLF
TGINNLDEGFIFTDATIQNDTISDQSQRVSQFDRLLAGLFPYITSEERQAVAKQYPISDA
PSKGNTFSRISAVIADSTFVCPTYWTAEAFGSSAHKGLFDYAPAHATDNSYYIGSIWNG
KKSVSSVQSF D GALGGFIETFNPNNAANKTINPYWPTFD SGKQLLFNTTRDTLSPADP
RIVETSSLTDGTSQTKCDFWRGSISVNAGL*

FIG. 10

Amino acid composition from 1 to 572
TRN 2-1738 RHODOSPORIDIUM ESTERASE cDNA

Total Percent

A	67	11.7
C	7	1.2
D	25	4.4
E	16	2.8
F	35	6.1
G	49	8.6
H	9	1.6
I	21	3.7
K	25	4.4
L	48	8.4
M	4	0.7
N	35	6.1
P	31	5.4
Q	26	4.5
R	16	2.8
S	52	9.1
T	43	7.5
V	32	5.6
W	10	1.7
Y	21	3.7
Acidic	41	7.2
Basic	41	7.2
Charged	82	14.3
Net charge	0	0.0
Hydrophobic	136	23.8
Residues	572	
MW	61334	

FIG. 11